

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of claims

1. (Currently Amended) A *cis*-acting nucleotide sequence which is capable of rendering the removal of introns from a precursor transcript encoded by any gene, which gene harbors at least one such *cis*-acting nucleotide sequence, occurring during the production of mRNA of said gene, dependent upon activation of a *trans*-acting factor, said *trans*-acting factor being the RNA-activated protein kinase (PKR) which is capable of phosphorylating the α -subunit of eukaryotic initiation factor 2, and wherein said *cis*-acting nucleotide sequence is derived from the 3' untranslated region of the human tumor necrosis factor α gene (TNF- α -3'UTR) and consists essentially of a sequence selected from the group consisting of SEQ ID NO:1, and SEQ ID NO:2, ~~and biologically functional fragments, derivatives, mutants and homologues of the nucleotide sequence as denoted by SEQ ID NO:1 or SEQ ID NO:2.~~

2. (Cancelled)

3. (Cancelled)

4. (Currently Amended) The *cis*-acting nucleotide sequence according to claim 1 wherein said *cis*-acting nucleotide sequence is derived from the 3' untranslated region of the human tumor necrosis factor α gene (TNF- α -3'UTR) and consists ~~essentially of a sequence selected from the group consisting of~~

a) the nucleotide sequence ~~substantially as~~ denoted by SEQ ID NO:1; ~~and~~

~~b) — biologically functional fragments, derivatives, mutants and
homologues of the nucleotide sequence as denoted by SEQ ID NO:1.~~

5. (Currently Amended) The *cis*-acting nucleotide sequence according to claim 1 wherein said *cis*-acting nucleotide sequence is derived from the 3' untranslated region of the human tumor necrosis factor α gene (TNF- α -3'UTR) and consists ~~essentially of a sequence selected from the group consisting of~~

- a) the nucleotide sequence as denoted by SEQ ID NO:2; ~~and~~
- ~~b) — biologically functional fragments, derivatives, mutants and homologues of
the nucleotide sequence as denoted by SEQ ID NO:2.~~

6. (Currently Amended) The *cis*-acting nucleotide sequence according to claim 5 wherein said gene encodes a protein selected from the group consisting of enzymes, hormones, growth factors, cytokines, structural proteins, industrially applicable proteins, agriculturally applicable proteins, a protein which is a therapeutic product, a protein which is an agricultural product, and a protein which is an industrially applicable product.

7. (Currently Amended) A DNA construct comprising:-

- a) a gene which contains at least one intron;
- b) a *cis*-acting nucleotide sequence which is capable of rendering the removal of introns from a precursor transcript encoded by said gene, which gene includes at least one such *cis*-acting nucleotide sequence, occurring during the production of mRNA of said gene, dependent upon activation of a *trans*-acting factor, wherein said *trans*-acting factor being the RNA-activated protein kinase

(PKR) which is capable of phosphorylating the α -subunit of eukaryotic initiation

factor 2, operably linked to said gene; and

c) optionally further comprising additional control, promoting and regulatory elements,

and wherein said *cis*-acting nucleotide sequence is derived from the 3' untranslated region of the human tumor necrosis factor α gene (TNF- α -3'UTR) and consists ~~essentially~~ of a sequence selected from the group consisting of SEQ ID NO:1, and SEQ ID NO:2, ~~and biologically functional fragments, derivatives, mutants and homologues of the nucleotide sequence as denoted by SEQ ID NO:1 or SEQ ID NO:2.~~

8. (Currently Amended) The DNA construct according to claim 7 wherein said *cis*-acting nucleotide sequence is derived from the 3' untranslated region of the human tumor necrosis factor α gene (TNF- α -3'UTR) and consists ~~essentially of a sequence selected from the group consisting of~~

a) the nucleotide sequence as denoted by SEQ ID NO:1; ~~and~~

b) ~~biologically functional fragments, derivatives, mutants and~~

~~homologues of the nucleotide sequence as denoted by SEQ ID NO:1.~~

9. (Currently Amended) The DNA construct according to claim 7 wherein said *cis*-acting nucleotide sequence is derived from the 3' untranslated region of the human tumor necrosis factor α gene (TNF- α -3'UTR) and consists ~~essentially of a sequence selected from the group consisting of~~

a) the nucleotide sequence as denoted by SEQ ID NO:2; ~~and~~

b) ~~biologically functional fragments, derivatives, mutants and homologues of~~
~~the nucleotide sequence as denoted by SEQ ID NO:2.~~

10. (Previously Presented) A DNA construct according to any one of claims 7, 8 or 9 wherein said control, promoting and regulatory elements are suitable transcription promoters, transcription enhancers and mRNA destabilizing elements.

11. (Previously Presented) The DNA construct according to claim 7, wherein said gene which contains at least one intron, encodes a protein selected from the group consisting of enzymes, hormones, growth factors, cytokines, structural proteins, industrially applicable proteins, agriculturally applicable proteins, a protein which is a therapeutic product, protein which is an agricultural product, and a protein which is an industrially applicable product.

12. (Previously Presented) The DNA construct according to claim 11 wherein said nucleotide sequence is contained within an exon of said gene.

13. (Currently Amended) The DNA construct according to claim 11 wherein said nucleotide sequence is ~~contained~~ inserted within an intron of said gene.

14. (Previously Presented) The DNA construct according to claim 13 wherein said gene is the human TNF- α gene.

15. (Previously Presented) The DNA construct according to claim 14 being the plasmid pTNF- α , in which said *cis*-acting element is contained within an exon of the human TNF- α gene.

16. (Previously Presented) The DNA construct according to claim 15 being the plasmid pTNF- α (3'UTR- α EP).

17. (Previously Presented) The DNA construct according to claim 7 wherein said gene is the human TNF- β gene.

18. (Previously Presented) The DNA construct according to claim 17 in which said *cis*-acting element is contained within an exon of the human TNF- β gene.

19. (Previously Presented) The DNA construct according to claim 18 being the plasmid pTNF- β (3'UTR- α).

20. (Previously Presented) The DNA construct according to claim 18 being the plasmid pTNF- β (3'UTR- α EP).

21. (Cancelled)

22. (Previously Presented) The DNA construct according to claim 14 wherein the DNA construct is pTNF α (Δ 3'UTR)i3EP.

23. (Previously Presented) A vector comprising the *cis*-acting nucleotide sequence according to claim 1 or the DNA construct according to claim 7 and a suitable DNA carrier, capable of transfecting a host cell with said *cis*-acting nucleotide sequence.

24. (Previously Presented) The vector according to claim 23 optionally further comprising additional expression, control, promoting and regulatory elements operably linked thereto.

25. (Previously Presented) The vector according to claim 24 wherein said carrier is salmon sperm DNA.

26. (Previously Presented) The vector according to claim 24 wherein said carrier is viral DNA.

27. (Previously Presented) A host cell transfected with the DNA construct according to claim 22.

28. (Previously Presented) A host cell transfected with the vector according to claim 23.

29. (Original) A host cell according to claim 27 or 28 being a eukaryotic or yeast cell.

30. (Previously Presented) The host cell according to claim 29 being a mammalian hemopoietic cell, fibroblast, epithelial cell, or lymphocyte.

31. (Previously Presented) The host cell according to claim 27 wherein said eukaryotic cell is the baby hamster kidney (BHK-21) cell line or the Chinese hamster ovary (CHO) cell line.

32-46. (Cancelled)

47. (Currently Amended) A ~~pharmaceutical composition comprising as active ingredient a therapeutically effective amount of~~ the expression vectors vector according to claim 23 ~~or of transformed host cells according to claim 30.~~

48. (Currently Amended) A method of producing a ~~recombinant therapeutic or industrially or agriculturally applicable~~ protein comprising the steps of:

- a) ~~providing the DNA construct according to claim 7 or the expression vector according to claim 23 wherein said gene encodes said protein;~~
- b) ~~transfecting a host cell with the~~ a DNA construct or expression vector provided in
(a) to give a host cell capable of expressing said protein in substantial amount wherein said DNA construct comprises a) a gene which contains at least one intron, wherein said gene encodes said protein; b) a cis-acting nucleotide sequence which is capable of rendering the removal of introns from a precursor transcript encoded by said gene, which gene includes at least one such cis-acting nucleotide sequence, occurring during the production of mRNA of said gene, dependent upon activation of a trans-acting factor, wherein said trans-acting factor being the

RNA-activated protein kinase (PKR) which is capable of phosphorylating the α -subunit of eukaryotic initiation factor 2, operably linked to said gene; and c) optionally further comprising additional control, promoting and regulatory elements, and wherein said *cis*-acting nucleotide sequence is derived from the 3' untranslated region of the human tumor necrosis factor α gene (TNF- α -3'UTR) and consists of a sequence selected from the group consisting of SEQ ID NO:1, and SEQ ID NO:2; and

[[c)] b) culturing the cells obtained in [[(b)] (a) under ~~suitable~~ culture conditions amenable to express said protein; and

[[d)] c) isolating said protein from the cell culture obtained in [[(c)] (b).

49. (Currently Amended) A method of producing a ~~recombinant therapeutic or industrially or agriculturally applicable~~ protein comprising ~~the steps of~~:

- a) providing host cells transfected with ~~the~~ a DNA construct ~~according to claim 7 or the expression vector according to claim 23 wherein said gene encodes said protein~~, which are capable of expressing said protein ~~in substantial amount~~, wherein said DNA construct comprises a) a gene which contains at least one intron, wherein said gene encodes said protein; b) a *cis*-acting nucleotide sequence which is capable of rendering the removal of introns from a precursor transcript encoded by said gene, which gene includes at least one such *cis*-acting nucleotide sequence, occurring during the production of mRNA of said gene, dependent upon activation of a *trans*-acting factor, wherein said *trans*-acting factor being the RNA-activated protein kinase (PKR) which is capable of phosphorylating the α -subunit of eukaryotic initiation factor 2, operably linked to

said gene; and c) optionally further comprising additional control, promoting and regulatory elements, and wherein said *cis*-acting nucleotide sequence is derived from the 3' untranslated region of the human tumor necrosis factor α gene (TNF- α -3'UTR) and consists of a sequence selected from the group consisting of SEQ ID NO:1, and SEQ ID NO:2;

b) culturing the cells provided in (a) under ~~suitable~~ culture conditions amenable to express said protein; and

c) isolating said protein from the cell culture obtained in (b).

50. (Cancelled)

51. (New) A composition comprising the host cell according to claim 30.

52. (New) A method of producing a protein comprising:

a) transfecting a host cell with an expression vector to produce a host cell capable of expressing said protein, wherein said expression vector is selected from the group consisting of

(1) a *cis*-acting nucleotide sequence which is capable of rendering the removal of introns from a precursor transcript encoded by any gene, which gene harbors at least one such *cis*-acting nucleotide sequence, occurring during the production of mRNA of said gene, dependent upon activation of a *trans*-acting factor, said *trans*-acting factor being the RNA-activated protein kinase (PKR) which is capable of phosphorylating the α -subunit of eukaryotic initiation factor 2, and wherein said *cis*-acting nucleotide sequence is derived from the 3' untranslated

region of the human tumor necrosis factor α gene (TNF- α -3'UTR) and consists of a sequence of SEQ ID NO:1 or SEQ ID NO:2; and

(2) a DNA construct comprising (A) a gene which contains at least one intron, wherein said gene encodes said protein; (B) a *cis*-acting nucleotide sequence which is capable of rendering the removal of introns from a precursor transcript encoded by said gene, which gene includes at least one such *cis*-acting nucleotide sequence, occurring during the production of mRNA of said gene, dependent upon activation of a *trans*-acting factor, wherein said *trans*-acting factor being the RNA-activated protein kinase (PKR) which is capable of phosphorylating the α -subunit of eukaryotic initiation factor 2, operably linked to said gene; and (C) optionally further comprising additional control, promoting and regulatory elements, and wherein said *cis*-acting nucleotide sequence is derived from the 3' untranslated region of the human tumor necrosis factor α gene (TNF- α -3'UTR) and consists of a sequence of SEQ ID NO:1 or SEQ ID NO:2, and a suitable DNA carrier;

- c) culturing the cells obtained in (b) under culture conditions amenable to express said protein; and
- d) isolating said protein from the cell culture obtained in (c).

53. (New) A method of producing a protein comprising:

- a) providing host cells transfected with an expression vector to produce a host cell capable of expressing said protein, wherein said expression vector is selected from the group consisting of

(1) a *cis*-acting nucleotide sequence which is capable of rendering the removal of introns from a precursor transcript encoded by any gene, which gene harbors at least one such *cis*-acting nucleotide sequence, occurring during the production of mRNA of said gene, dependent upon activation of a *trans*-acting factor, said *trans*-acting factor being the RNA-activated protein kinase (PKR) which is capable of phosphorylating the α -subunit of eukaryotic initiation factor 2, and wherein said *cis*-acting nucleotide sequence is derived from the 3' untranslated region of the human tumor necrosis factor α gene (TNF- α -3'UTR) and consists of a sequence of SEQ ID NO:1 or SEQ ID NO:2; and

(2) a DNA construct comprising (A) a gene which contains at least one intron, wherein said gene encodes said protein; (B) a *cis*-acting nucleotide sequence which is capable of rendering the removal of introns from a precursor transcript encoded by said gene, which gene includes at least one such *cis*-acting nucleotide sequence, occurring during the production of mRNA of said gene, dependent upon activation of a *trans*-acting factor, wherein said *trans*-acting factor being the RNA-activated protein kinase (PKR) which is capable of phosphorylating the α -subunit of eukaryotic initiation factor 2, operably linked to said gene; and (C) optionally further comprising additional control, promoting and regulatory elements, and wherein said *cis*-acting nucleotide sequence is derived from the 3' untranslated region of the human tumor necrosis factor α gene (TNF- α -3'UTR) and consists of a sequence of SEQ ID NO:1 or SEQ ID NO:2, and a suitable DNA carrier,

- b) culturing the cells provided in (a) under culture conditions amenable to express said protein; and
- c) isolating said protein from the cell culture obtained in (b).